

Claims

5 1. A genetically engineered cell which expresses a polyketide synthase (PKS) gene cluster in its native, nontransformed state, said genetically engineered cell substantially lacking the entire native PKS gene cluster.

10 2. The genetically engineered cell of claim 1, wherein the cell is a procaryotic cell.

15 3. The genetically engineered cell of claim 2, wherein the cell is an actinomycete.

4. The genetically engineered cell of claim 3, wherein the cell is an actinomycete of the genus *Streptomyces*.

20 5. The genetically engineered cell of claim 4, wherein the cell is *Streptomyces coelicolor*.

25 6. The genetically engineered cell of claim 5, wherein the cell substantially lacks the entire native actinorhodin PKS gene cluster.

7. The genetically engineered cell of claim 6, wherein the cell is equivalent to strain CH999.

30 8. The genetically engineered cell of claim 1, wherein the cell comprises:

(a) a replacement PKS gene cluster which encodes a PKS capable of catalyzing the synthesis of a polyketide; and

35

(b) one or more control sequences operatively linked to said PKS gene cluster, whereby the genes in said gene cluster can be transcribed and translated in the genetically engineered cell,

5 with the proviso that when the replacement PKS gene cluster comprises an entire PKS gene set, at least one of the PKS genes or control elements is heterologous to the cell.

10 9. The genetically engineered cell of claim 5, wherein the cell comprises

(a) a replacement PKS gene cluster which encodes a PKS capable of catalyzing the synthesis of a polyketide, and

15 (b) one or more control sequences operatively linked to said PKS gene cluster, whereby the genes in said gene cluster can be transcribed and translated in the genetically engineered cell,

20 with the proviso that when the replacement PKS gene cluster comprises an entire PKS gene set, at least one of the PKS genes or control elements is heterologous to the cell.

25 10. The genetically engineered cell of claim 8, wherein the replacement PKS gene cluster comprises a first gene encoding a PKS ketosynthase and a PKS acyltransferase active site (KS/AT), a second gene encoding a PKS chain length determining factor (CLF), and a third gene encoding a PKS acyl carrier protein (ACP).

30 11. The genetically engineered cell of claim 9, wherein the replacement PKS gene cluster comprises a first gene encoding a PKS ketosynthase and a PKS acyltransferase active site (KS/AT), a second gene

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encoding a PKS chain length determining factor (CLF), and a third gene encoding a PKS acyl carrier protein (ACP).

5 12. The genetically engineered cell of claim 10, wherein the replacement PKS gene cluster further comprises a fourth gene encoding a PKS ketoreductase (KR).

10 13. The genetically engineered cell of claim 11, wherein the replacement PKS gene cluster further comprises a fourth gene encoding a PKS ketoreductase (KR).

15 14. The genetically engineered cell of claim 12, wherein the replacement PKS gene cluster further comprises a fifth gene encoding a PKS cyclase (CYC).

20 15. The genetically engineered cell of claim 13, wherein the replacement PKS gene cluster further comprises a fifth gene encoding a PKS cyclase (CYC).

25 16. The genetically engineered cell of claim 14, wherein the replacement PKS gene cluster further comprises a sixth gene encoding a PKS dehydratase.

17. The genetically engineered cell of claim 15, wherein the replacement PKS gene cluster further comprises a sixth gene encoding a PKS dehydratase.

30 18. The genetically engineered cell of claim 10, wherein the PKS KS/AT gene is derived from a PKS KS/AT gene selected from the group consisting of an actinorhodin KS/AT gene, a granaticin KS/AT gene, a tetracenomycin KS/AT gene, a frenolicin B KS/AT gene, an  
35 oxytetracycline KS/AT gene, and a griseusin KS/AT gene.

19. The genetically engineered cell of claim 11, wherein the PKS KS/AT gene is derived from a PKS KS/AT gene selected from the group consisting of an actinorhodin KS/AT gene, a granaticin KS/AT gene, a tetracenomycin KS/AT gene, a frenolicin B KS/AT gene, an oxytetracycline KS/AT gene, and a griseusin KS/AT gene.

20. The genetically engineered cell of claim 10, wherein the PKS CLF gene is derived from a PKS CLF gene selected from the group consisting of an actinorhodin CLF gene, a granaticin CLF gene, a tetracenomycin CLF gene, a frenolicin B CLF gene, an oxytetracycline CLF gene, and a griseusin CLF gene.

21. The genetically engineered cell of claim 11, wherein the PKS CLF gene is derived from a PKS CLF gene selected from the group consisting of an actinorhodin CLF gene, a granaticin CLF gene, a tetracenomycin CLF gene, a frenolicin B CLF gene, an oxytetracycline CLF gene, and a griseusin CLF gene.

22. The genetically engineered cell of claim 10, wherein the PKS ACP gene is derived from a PKS ACP gene selected from the group consisting of an actinorhodin ACP gene, a granaticin ACP gene, a tetracenomycin ACP gene, a frenolicin B ACP gene, an oxytetracycline ACP gene, and a griseusin ACP gene.

23. The genetically engineered cell of claim 11, wherein the PKS ACP gene is derived from a PKS ACP gene selected from the group consisting of an actinorhodin ACP gene, a granaticin ACP gene, a tetracenomycin ACP gene, a frenolicin B ACP gene, an oxytetracycline ACP gene, and a griseusin ACP gene.

24. The genetically engineered cell of claim  
12, wherein the PKS KR gene is derived from a PKS KR gene  
selected from the group consisting of an actinorhodin KR  
gene, a granaticin KR gene, a tetracenomycin KR gene, a  
5 frenolicin B KR gene, an oxytetracycline KR gene, and a  
griseusin KR gene.

25. The genetically engineered cell of claim  
13, wherein the PKS KR gene is derived from a PKS KR gene  
10 selected from the group consisting of an actinorhodin KR  
gene, a granaticin KR gene, a tetracenomycin KR gene, a  
frenolicin B KR gene, an oxytetracycline KR gene, and a  
griseusin KR gene.

15 26. The genetically engineered cell of claim  
10, wherein each of said first, second and third genes  
are contained in separate expression cassettes.

20 27. The genetically engineered cell of claim  
11, wherein each of said first, second and third genes  
are contained in separate expression cassettes.

25 28. The genetically engineered cell of claim  
26, wherein the separate expression cassettes are present  
in a single vector.

30 29. The genetically engineered cell of claim  
27, wherein the separate expression cassettes are present  
in a single vector.

30 30. The genetically engineered cell of claim  
26, wherein the separate expression cassettes are present  
in two or more vectors.

31. The genetically engineered cell of claim 27, wherein the separate expression cassettes are present in two or more vectors.

5 32. The genetically engineered cell of claim 8, wherein the replacement gene cluster comprises a first gene encoding a PKS acyltransferase (AT), a second gene encoding a PKS ketoacyl carrier protein synthase (KS), a third gene encoding a PKS acyl carrier protein (ACP), a  
10 fourth gene encoding a PKS ketoreductase (KR), a fifth gene encoding a PKS dehydratase (DH), a sixth gene encoding a PKS enoyl reductase (ER), and a seventh gene encoding a thioesterase (TE).

15 33. The genetically engineered cell of claim 32, wherein the genes are derived from the 6-deoxyerythronolide B synthase gene cluster.

20 34. The genetically engineered cell of claim 9, wherein the replacement gene cluster comprises a first gene encoding a PKS acyltransferase (AT), a second gene encoding a PKS ketoacyl carrier protein synthase (KS), a third gene encoding a PKS acyl carrier protein (ACP), a fourth gene encoding a PKS ketoreductase (KR), a fifth  
25 gene encoding a PKS dehydratase (DH), a sixth gene encoding a PKS enoyl reductase (ER), and a seventh gene encoding a thioesterase (TE).

30 35. The genetically engineered cell of claim 34, wherein the genes are derived from the 6-deoxyerythronolide B synthase gene cluster.

36. A method for producing a recombinant polyketide comprising:

(a) providing a population of cells according to claim 8; and

5 (b) culturing the population of cells under conditions whereby the replacement PKS gene cluster present in the cells, is expressed.

10 37. A method for producing a recombinant polyketide comprising:

(a) providing a population of cells according to claim 9; and

15 (b) culturing the population of cells under conditions whereby the replacement PKS gene cluster, present in the cells, is expressed.

38. A method for producing a recombinant polyketide comprising:

20 (a) providing a population of cells according to claim 10; and

(b) culturing the population of cells under conditions whereby the replacement PKS gene cluster, present in the cells, is expressed.

25 39. A method for producing a recombinant polyketide comprising:

(a) providing a population of cells according to claim 11; and

30 (b) culturing the population of cells under conditions whereby the replacement PKS gene cluster, present in the cells, is expressed.

40. A method for producing a recombinant polyketide comprising:

5 a. inserting a first portion of a replacement PKS gene cluster into a donor plasmid and inserting a second portion of a replacement PKS gene cluster into a recipient plasmid, wherein the first and second portions collectively encode a complete replacement PKS gene cluster, and further wherein:

10 i. the donor plasmid expresses a gene which encodes a first selection marker and is capable of replication at a first, permissive temperature and incapable of replication at a second, non-permissive temperature;

15 ii. the recipient plasmid expresses a gene which encodes a second selection marker; and

20 iii. the donor plasmid comprises regions of DNA complementary to regions of DNA in the recipient plasmid, such that homologous recombination can occur between the first portion of the replacement PKS gene cluster and the second portion of the replacement gene cluster, whereby a complete replacement gene cluster can be generated;

25 b. transforming the donor plasmid and the recipient plasmid into a host cell and culturing the transformed host cell at the first, permissive temperature and under conditions which allow the growth of host cells which express the first and/or the second selection markers, to generate a first population of cells;

30 c. culturing the first population of cells at the second, non-permissive temperature and under conditions which allow the growth of cells which express the first and/or the second selection markers, to generate a second population of cells which includes host



cells which contain a recombinant plasmid comprising a complete PKS replacement gene cluster;

d. transferring the recombinant plasmid from the second population of cells into the genetically engineered cell of claim 1 to generate transformed  
5 genetically engineered cells; and

e. culturing the transformed genetically engineered cells under conditions whereby the replacement PKS gene cluster present in the cells is expressed.

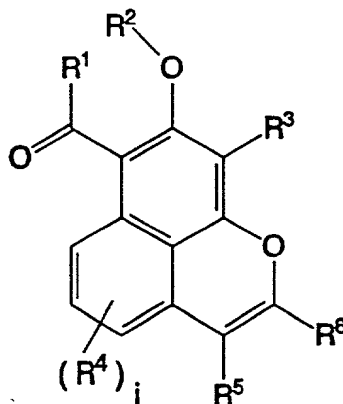
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41. The method of claim 40, wherein the method further comprises after step (c) culturing the second population of cells at the first, permissive temperature and under conditions which allow the growth of cells  
15 which express the first selection marker.

42. The method of claim 40, wherein the first and second portions of the replacement PKS gene cluster are derived from the 6-deoxyerythronolide B synthase gene  
20 cluster.

43. A polyketide compound having the structural formula

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wherein:

5           R<sup>1</sup> is selected from the group consisting of  
hydrogen and lower alkyl and R<sup>2</sup> is selected from the  
group consisting of hydrogen, lower alkyl and lower alkyl  
ester, or wherein R<sup>1</sup> and R<sup>2</sup> together form a lower  
alkylene bridge optionally substituted with one to four  
10 hydroxyl or lower alkyl groups;

          R<sup>3</sup> and R<sup>5</sup> are independently selected from the  
group consisting of hydrogen, halogen, lower alkyl, lower  
alkoxy, amino, lower alkyl mono- or di-substituted amino  
and nitro;

15           R<sup>4</sup> is selected from the group consisting of  
halogen, lower alkyl, lower alkoxy, amino, lower alkyl  
mono- or di-substituted amino and nitro;

          R<sup>6</sup> is selected from the group consisting of  
hydrogen, lower alkyl, and -CHR<sup>7</sup>-(CO)R<sup>8</sup> where R<sup>7</sup> and R<sup>8</sup>  
20 are independently selected from the group consisting of  
hydrogen and lower alkyl; and

i is 1, 2 or 3.

44. The compound of claim 43, wherein:

25           R<sup>1</sup> is lower alkyl;  
          R<sup>2</sup>, R<sup>3</sup> and R<sup>5</sup> are hydrogen;  
          R<sup>6</sup> is -CHR<sup>7</sup>-(CO)-R<sup>8</sup>; and  
          i is 0.

30           45. The compound of claim 44, wherein R<sup>1</sup> is  
methyl and R<sup>6</sup> is -CH<sub>2</sub>-(CO)-CH<sub>3</sub>.

46. The compound of claim 43, wherein:

35           R<sup>1</sup> and R<sup>6</sup> are lower alkyl;  
          R<sup>2</sup>, R<sup>3</sup> and R<sup>5</sup> are hydrogen; and

i is 0.

47. The compound of claim 46, wherein  $R^1$  and  $R^6$  are methyl.

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48. The compound of claim 43, wherein  $R^1$  and  $R^2$  are linked together to form a lower alkylene bridge -  $CHR^9-CHR^{10}$  wherein  $R^9$  and  $R^{10}$  are independently selected from the group consisting of hydrogen, hydroxyl and lower alkyl.

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49. The compound of claim 43, wherein  $R^1$  and  $R^2$  are linked together to form a lower alkylene bridge -  $CH_2-CHOH-$ .

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50. The compound of claim 49, wherein:  
 $R^3$  and  $R^5$  are hydrogen; and  
i is 0.

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51. The compound of claim 50, wherein  $R^6$  is  $-CHR^7-(CO)-R^8$  where  $R^8$  is hydrogen or lower alkyl.

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52. The compound of claim 51, wherein  $R^6$  is  $-CH_2-(CO)-CH_3$ .

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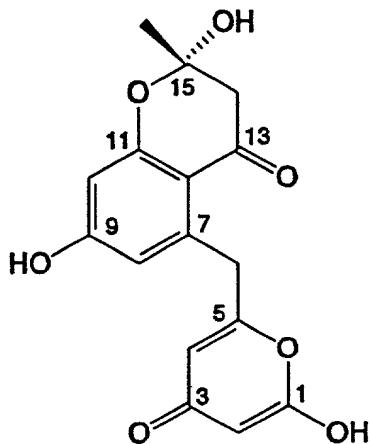
53. The compound of claim 50, wherein  $R^6$  is lower alkyl.

54. The compound of claim 53 wherein  $R^6$  is methyl.

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55. A polyketide compound having the structural formula

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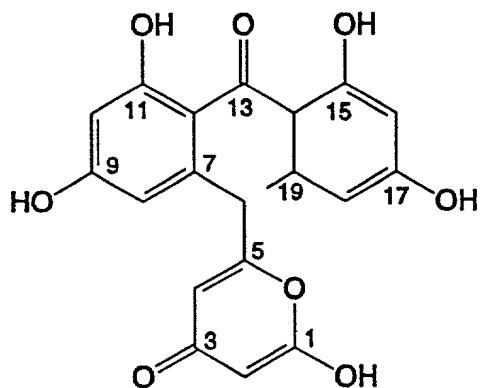


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56. A polyketide compound having the structural formula

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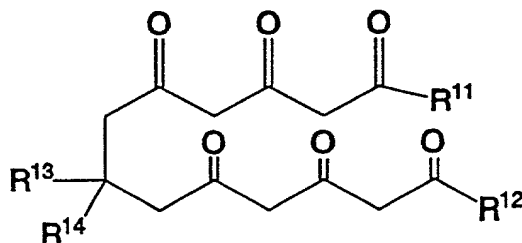


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57. A polyketide compound formed by catalytic cyclization of an enzyme-bound ketide having the structure



wherein:

15  $R^{11}$  is selected from the group consisting of methyl,  $-\text{CH}_2(\text{CO})\text{CH}_3$  and  $-\text{CH}_2(\text{CO})\text{CH}_2(\text{CO})\text{CH}_3$ ;

$R^{12}$  is selected from the group consisting of  $-\text{S-E}$  and  $-\text{CH}_2(\text{CO})-\text{S-E}$ , wherein E represents a polyketide synthase produced by the genetically engineered cell of claim 8; and

20 one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl, or  $R^{13}$  and  $R^{14}$  together represent carbonyl.

58. The compound of claim 57, wherein  $R^{11}$  is methyl and  $R^{12}$  is  $-\text{CH}_2(\text{CO})-\text{S-E}$ .

25 59. The compound of claim 57, wherein  $R^{11}$  is  $-\text{CH}_2(\text{CO})\text{CH}_3$  and  $R^{12}$  is  $-\text{S-E}$ .

30 60. The compound of claim 57, wherein  $R^{11}$  is  $-\text{CH}_2(\text{CO})\text{CH}_3$  and  $R^{12}$  is  $-\text{CH}_2(\text{CO})-\text{S-E}$ .

61. The compound of claim 57, wherein  $R^{11}$  is  $-\text{CH}_2(\text{CO})\text{CH}_2(\text{CO})\text{CH}_3$  and  $R^{12}$  is  $-\text{CH}_2(\text{CO})-\text{S-E}$ .

62. The compound of claim 58, wherein one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl.

5 63. The compound of claim 59, wherein one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl.

64. The compound of claim 60, wherein one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl.

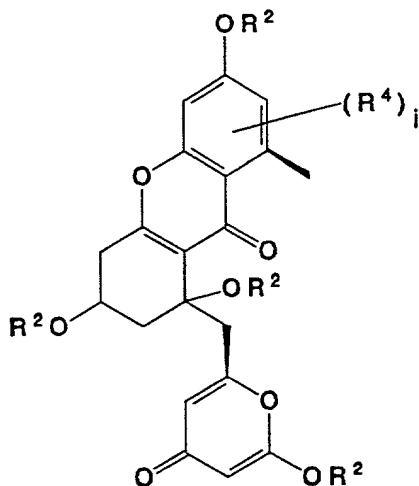
10 65. The compound of claim 61, wherein one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl.

66. The compound of claim 62, wherein  $R^{13}$  and  $R^{14}$  together form a carboxyl moiety.

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67. A polyketide compound having the structural formula

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wherein;

the  $R^2$  moieties are independently selected from the group consisting of hydrogen, lower alkyl and lower alkyl esters;

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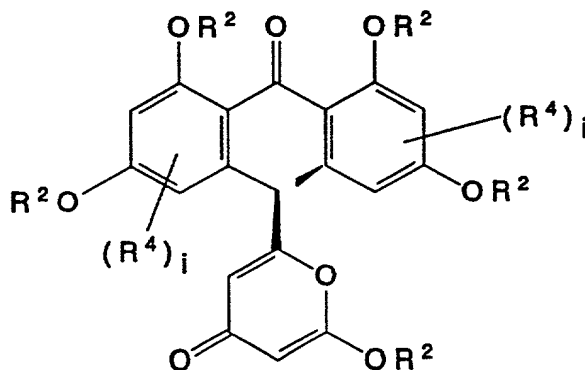
$R^4$  is selected from the group consisting of halogen, lower alkyl, lower alkoxy, amino, lower alkyl mono- or di-substituted amino and nitro; and  $i$  is 0, 1 or 2.

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68. The compound of claim 67, wherein  $R^2$  is hydrogen and  $i$  is 0.

69. A polyketide compound having the structural formula

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wherein:

the  $R^2$  moieties are independently selected from the group consisting of hydrogen, lower alkyl and lower alkyl esters;

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$R^4$  is selected from the group consisting of halogen, lower alkyl, lower alkoxy, amino, lower alkyl mono- or di-substituted amino and nitro; and

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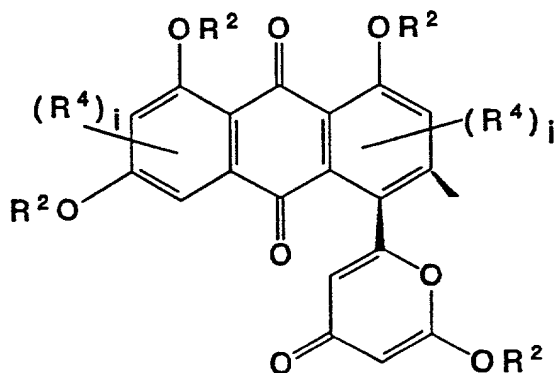
$i$  is 0, 1 or 2.

70. The compound of claim 69, wherein  $R^2$  is hydrogen and  $i$  is 0.

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71. A polyketide compound having the structural formula

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wherein:

the R<sup>2</sup> moieties are independently selected from the group consisting of hydrogen, lower alkyl and lower alkyl esters;

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R<sup>4</sup> is selected from the group consisting of halogen, lower alkyl, lower alkoxy, amino, lower alkyl mono- or di-substituted amino and nitro; and

i is 0, 1 or 2.

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72. The compound of claim 71, wherein R<sup>2</sup> is hydrogen and i is 0.

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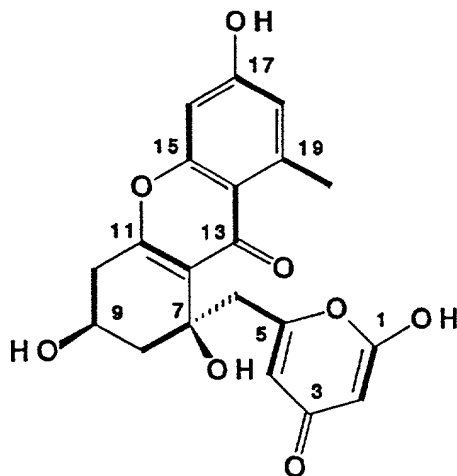
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73. A polyketide compound having the structural formula

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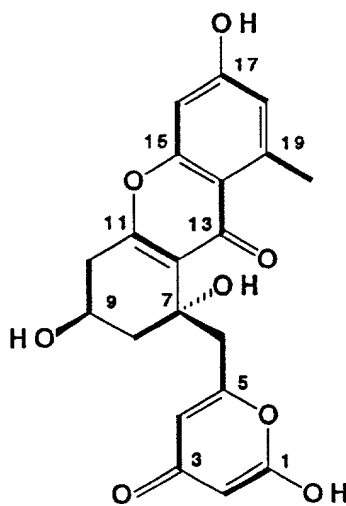


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74. A polyketide compound having the structural formula

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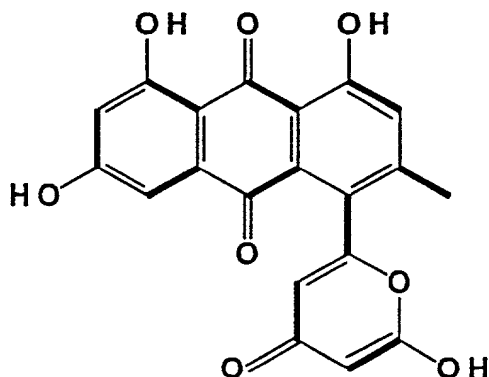


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75. A polyketide compound having the structural formula

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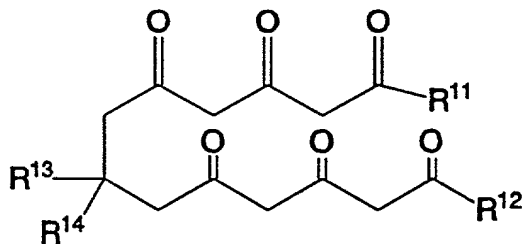


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76. A method for producing an aromatic polyketide, comprising effecting cyclization of an enzyme-bound ketide having the structure

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wherein:

30  $R^{11}$  is selected from the group consisting of methyl,  $-\text{CH}_2(\text{CO})\text{CH}_3$  and  $-\text{CH}_2(\text{CO})\text{CH}_2(\text{CO})\text{CH}_3$ ;

$R^{12}$  is selected from the group consisting of  $-\text{S-E}$  and  $-\text{CH}_2(\text{CO})-\text{S-E}$ , wherein E represents a polyketide synthase produced by the genetically engineered cell of claim 8; and

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one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl, or  $R^{13}$  and  $R^{14}$  together represent carbonyl, wherein cyclization is induced by said polyketide synthase.

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77. The compound of claim 57, wherein  $R^{11}$  is methyl and  $R^{12}$  is  $-\text{CH}_2(\text{CO})-\text{S}-\text{E}$ .

78. The compound of claim 57, wherein  $R^{11}$  is  $-\text{CH}_2(\text{CO})\text{CH}_3$  and  $R^{12}$  is  $-\text{S}-\text{E}$ .

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79. The compound of claim 57, wherein  $R^{11}$  is  $-\text{CH}_2(\text{CO})\text{CH}_3$  and  $R^{12}$  is  $-\text{CH}_2(\text{CO})-\text{S}-\text{E}$ .

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80. The method of claim 77, wherein  $R^{11}$  is  $-\text{CH}_2(\text{CO})\text{CH}_2(\text{CO})\text{CH}_3$  and  $R^{12}$  is  $-\text{CH}_2(\text{CO})-\text{S}-\text{E}$ .

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81. The method of claim 77, wherein one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl.

82. The method of claim 78, wherein one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl.

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83. The method of claim 79, wherein one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl.

84. The method of claim 80, wherein one of  $R^{13}$  and  $R^{14}$  is hydrogen and the other is hydroxyl.

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85. The method of claim 80, wherein  $R^{13}$  and  $R^{14}$  together form a carboxyl moiety.

86. A polyketide produced by the method of claim 36.

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87. A polyketide produced by the method of claim 37.

5 88. A polyketide produced by the method of claim 38.

89. A polyketide produced by the method of claim 39.

10 90. A recombinant vector comprising:

(a) a DNA sequence comprising a modular replacement PKS gene cluster; and

15 (b) control elements that are operably linked to said DNA sequence whereby said DNA sequence can be transcribed and translated in a host cell and at least one of said control elements is heterologous to said nucleotide sequence.

20 91. The recombinant vector of claim 90, wherein the replacement gene cluster is the 6-deoxyerythronolide B synthase gene cluster.

92. The plasmid pCK7.

25 93. A host cell transformed with the vector of claim 90.

94. A host cell transformed with the vector of claim 91.

30 95. A host cell transformed with the vector of claim 92.